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Outbreak of Shiga toxin-producing *Escherichia coli* O157 linked with consumption of a fast-food product containing imported cucumbers, United Kingdom, August 2020

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ABSTRACT

Background: In August 2020, an outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 occurred in the United Kingdom. Whole genome sequencing revealed that these cases formed a genetically distinct cluster.

Methods: Hypotheses generated from case interviews were tested in analytical studies, and results informed environmental sampling and food chain analysis. A case–case study used non-outbreak ‘comparison’ STEC cases; a case–control study used a market research panel to recruit controls.

Results: A total of 36 cases were identified; all cases reported symptom onset between August 3 and August 16, 2020. The majority of cases (83%) resided in the Midlands region of England and in Wales. A high proportion of cases reported eating out, with one fast-food restaurant chain mentioned by 64% ($n = 23$) of cases. Both the case–case study (adjusted odds ratio (aOR) 31.8, 95% confidence interval (CI) 1.6–624.9) and the case–control study (aOR 9.19, 95% CI 1.0–82.8) revealed statistically significant results, showing that the consumption of a specific fast-food product was independently associated with infection.

Conclusions: Consumption of a specific fast-food product was a likely cause of this outbreak. The only ingredient specific to the product was cucumbers. The supply of cucumbers was immediately halted, and no further cases have been identified.

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Introduction

Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 are food-borne zoonotic bacteria that can cause gastroenteritis. STEC O157:

H7 are of significant public health concern in the United Kingdom due to the severity of symptoms (Bryne et al., 2015), which range from mild to bloody diarrhoea, with haemolytic uraemic syndrome (HUS), a life-threatening condition of the kidneys, occurring in a small proportion of cases (Launders et al., 2016a).

The principal reservoir for STEC O157:H7 is domestic ruminants (Blanco et al., 2001), primarily cattle, sheep, and goats. Human infections can occur through direct exposure to infected animals and their environment, the consumption of contaminated

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food/water, or human-to-human transmission (Byrne et al., 2014). Previous outbreaks of STEC O157:H7 in the United Kingdom have been found to be associated with the handling of raw leeks (Launders et al., 2016b) and the consumption of watercress (Jenkins et al., 2015), mixed salad leaves (Gobin et al., 2018), raw drinking milk (Treacy et al., 2019), and frozen beef burgers (Byrne et al., 2020), among others.

In the United Kingdom, STEC O157:H7 is the most commonly reported serogroup, with PT8 the most common phage type (PT). STEC are characterized by the presence of one or both Shiga toxin genes (*stx*): *stx1* (of which there are three subtypes, namely 1a, 1c, and 1d) and/or *stx2* (of which there are seven subtypes, *stx2a*–*stx2g*). The presence of *stx2*, specifically *stx2a*, is more likely to cause severe disease (Launders et al., 2016a,b; Brandal et al., 2015). In the United Kingdom, there are three lineages (I, II, and I/II) of STEC O157 and seven sub-lineages, Ia–c, IIa–c, and I/II (Dallman et al., 2015a).

On August 15, 2020, a cluster of five STEC O157 PT8 *stx1* + *stx2* were notified to Public Health England (PHE), all with a reported date of symptom onset between August 8 and August 10, 2020. Whole genome sequencing (WGS) revealed that these cases formed a genetically distinct cluster. An incident management team meeting was held on August 18 to coordinate the incident and guide appropriate epidemiological, microbiological, and food chain investigations (Figure 1).

Methods

Epidemiological methods

Case definitions

A confirmed outbreak case was a microbiological case of STEC O157:H7 *stx1a* + *stx2c* with a PHE Single Nucleotide Polymorphism (SNP) type 2.154.2186.4115.5439.5892.% (t5.5892), who was resident in the United Kingdom.

A probable outbreak case was a microbiological case of STEC O157:H7, PT8, *stx1* + *stx2*, with a date of onset or specimen date from August 1, 2020, awaiting WGS.

Enhanced surveillance and trawling questionnaires

Standardized enhanced surveillance questionnaires (ESQ) (PHE, 2019) were administered to all new case individuals with a STEC infection within 24 h of notification and reported to the National Enhanced Surveillance System for STEC infection (NESSS) database held by the Gastrointestinal Pathogens Unit. Analysis of the ESQs led to the design of a more detailed trawling questionnaire (TQ) for confirmed cases, focusing on all foods consumed both inside and outside of the home.

From August 21, 2020, the confirmed outbreak cases were contacted and asked to complete a TQ. The data from the ESQs and

TQs were combined to describe food exposures and to generate primary hypotheses about potential vehicles of transmission.

Case–case study

A case–case study was initiated on August 26, 2020 to test the initial hypotheses identified through the TQs (consumption of one specific fast-food product from restaurant chain A, lettuce and/or cucumbers), utilizing NESSS data. Outbreak cases that were available in NESSS (which only includes cases from England) and that met the confirmed case definition were compared to non-outbreak ‘comparison’ cases, which were (1) microbiologically confirmed cases of STEC O157; (2) infected with a different PT (not PT8) or PT8 with a different WGS profile and not associated with a known outbreak; (3) with a date of onset from July 1, 2020; (4) no travel outside the United Kingdom in the week before symptom onset; and (5) with an ESQ available in NESSS. No restrictions were placed on age or sex.

The data analysis was conducted in Stata 15. A univariate analysis was conducted to calculate the odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs). Any variable that was statistically significant in the univariate analysis (Chi-square P -value <0.05), had an OR >3, was a primary hypothesis, or was considered a potential confounder (age and sex) was included in a multivariable model. Any variable that had a Wald P -value >0.05 was considered for removal; each variable was then removed individually and a likelihood ratio test (LRT) performed to examine whether it improved the fit of the model (P < 0.05). Any variable that did not improve the fit of the model was further considered for removal; if the OR for any of the remaining variables changed by >20% on removal, it was considered a potential confounder and retained. The multivariable model was considered final when no further variables could be removed. Firth logistic regression was used to compute adjusted ORs (aORs) and 95% CIs in the final multivariable model, due to zero exposures in the ‘comparison’ group.

Case–control study

A case–control study was initiated on September 23, 2020 to further test the tiered hypotheses of association with eating out, eating at restaurant chain A, consumption of fast-food product X, and/or consumption of cucumber. A food exposure study-specific questionnaire was designed based upon these hypotheses. The sample size was calculated based on available data on lettuce consumption, assuming slight underreporting (due to recall bias): 45% in cases and 15% in controls. Assuming a significance level of 0.05 and power of 0.80, with a ratio of 3:1 controls to cases, a sample size of 26 cases and 78 controls was required. Inclusion criteria for controls were (1) age over 18 years; (2) had not experienced diarrhoea or vomiting, or been in close contact with someone with either symptom, and had not travelled outside the United Kingdom between July 27 and August 2, 2020.

Controls were recruited from October 2 to October 6, 2020 through a market research panel (Mook et al., 2018). Controls were frequency matched by sex, age (>50 years, <50 years), and broad geographic region (Midlands, Wales, and England excluding Midlands). Data on exposures for controls were collected for the period August 3 to August 9, 2020; this timeframe was chosen to reflect a similar exposure period to the cases.

The data analysis was conducted in Stata 15. Univariate analysis was conducted to calculate ORs and 95% CIs. Any variable that was statistically significant (Chi-square P < 0.05), had an OR >3, or was considered a potential confounder (age or sex) was included in the multivariable analysis, in a backwards, stepwise approach (full details under the case–case study methods). LRT was also used to explore interactions of food exposures and geographic region. Logistic regression was used to compute aORs and 95% CIs.

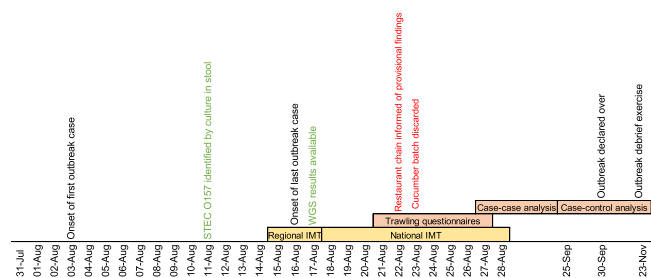


Figure 1. Timeline of the investigation and interventions; Shiga toxin-producing *Escherichia coli* O157:H7 outbreak, United Kingdom, August 2020.

Yellow: coordination and management of the outbreak investigation; orange: epidemiological investigations. Text in green: microbiological investigations; text in red: control measures. (IMT, incident management team).

Microbiological methods

Human samples

Isolates of *E. coli* O157 from community and hospitalized cases were referred to the Gastrointestinal Bacteria Reference Unit for confirmation and typing. On receipt, isolates were tested by PCR to determine the presence of the genes encoding Shiga toxin (*stx1* and/or *stx2*), phage-typed, and sequenced on the Illumina sequencing platform, as described previously (Khakhria et al., 1990; Jenkins et al., 2012; Dallman et al., 2015b). Sequences were analysed to determine the *stx* subtype (Ashton et al., 2015) and phylogenetic relationship between isolates to identify those that fell within the same five-SNP single linkage cluster, indicating that the isolates were likely to originate from the same source (Dallman et al., 2018; Jenkins et al., 2019). Sequences were deposited in the National Center for Biotechnology Information Sequence Read Archive under the BioProject PRJNA315192.

The Enterobase database (a publicly accessible database of genomes of several bacterial genera) was queried for isolates within a 50-SNP of the outbreak strain. An urgent enquiry was also posted on the Epidemic Intelligence Information System (EPIS) platform to determine whether any other countries had detected related strains or whether an increase in STEC O157:H7 *stx1* + *stx2* had been detected in other countries.

Food samples

Where available, samples of potential food sources/vehicles were obtained and tested at the PHE Food, Water and Environment (FW&E) laboratory in London. The FW&E laboratory tested all food samples using PHE Standard Method F17 based on BS EN ISO 16654:2001 Detection of *E. coli* O157 (ISO, 2001). The laboratory used by the international supplier tested all of their samples using the ISO 16654:2001 method as well as the international standard based on ISO/TS 13136:2012 (ISO, 2012).

Food chain investigations

The Food Standards Agency (FSA) co-ordinated food chain investigations with the establishments indicated by the initial epidemiological investigations, with a primary focus on restaurant chain A. A communication was raised via the Rapid Alert System for Food and Feed (RASFF) on August 24 to inform the European Union of the outbreak investigation.

Results

Epidemiology

Descriptive epidemiology

A total of 36 confirmed cases were identified as part of this outbreak, with the date of symptom onset ranging from August 3 to August 16, 2020 (Figure 2). Twenty-four cases were female (67%) and 12 were male (33%). Cases were between 13 and 60 years of age (median 26 years; interquartile range 19–32.5 years). Cases were distributed across England ($n = 27$) and Wales ($n = 9$), with the majority (58%) residing in the two Midlands regions ($n = 21$) (Figure 3). Clinical information was available for 33 of the cases: 13 were hospitalized and 25 reported bloody diarrhoea. There were no reports of HUS.

Enhanced surveillance and trawling questionnaires

Between August 23 and August 27, 2020, TQs were completed with 20 confirmed cases. A high proportion of cases had consumed food from restaurants and/or takeaways, with several reporting eating out multiple times in the 7 days prior to symptom onset. The most common establishment mentioned was restaurant chain A

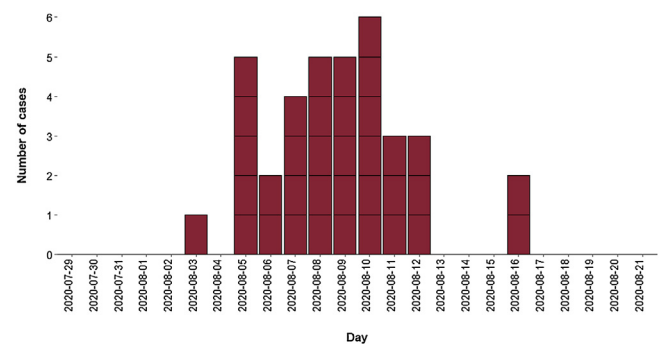


Figure 2. Epidemic curve of confirmed cases of Shiga toxin-producing *Escherichia coli* O157:H7 during the outbreak, United Kingdom, August 2020 ($n = 36$).

($n = 23$, 64%), with 16 reporting the consumption of fast-food product X (70%). A high proportion also reported eating processed chicken products ($n = 27$, 75%), lettuce ($n = 23$, 64%), and cucumber ($n = 17$, 47%). In total, 29 (81%) of the confirmed cases reported eating either cucumber (inside or outside the home) or the specific fast-food product X (which contained cucumber, chicken, lettuce, and sauce). No cases reported eating another similar fast-food product (product Y) available at the same restaurant chain, which contained a different chicken product, bacon, lettuce, tomato, and a different sauce, but no cucumber.

Case-case analysis

A total of 27 cases and 80 non-outbreak case ‘comparisons’ were included in this analysis. From the univariate analysis, there was evidence of an association between the consumption of raw vegetables, iceberg lettuce, fast-food product X, Indian takeaways and food from several other food establishments, and the odds of infection. In the multivariable analysis, there was strong evidence that the consumption of fast-food product X (aOR 31.79, 95% CI 1.62–624.89), an Indian takeaway (aOR 6.99, 95% CI 1.16–42.30), and raw vegetables (aOR 2.24, 95% CI 0.93–11.31) were independently associated with infection with the outbreak strain (Table 1).

Case-control analysis

A total of 25 cases and 85 controls were included in this analysis. The remaining 11 cases were not available to complete the study questionnaire. Single variable analysis was conducted to test the a priori tiered hypotheses (Table 2). The multivariable analysis indicated an association between outbreak cases and the consumption of fast-food product X (OR 9.19, 95% CI 1.02–82.8; $P = 0.048$) (Table 3).

Microbiology

Human cases

All isolates from the 36 confirmed outbreak cases fell within the same five-SNP single linkage cluster that belonged to lineage IIc. STEC O157:H7 lineage IIc is geographically dispersed across Europe (Dallman et al., 2021, accepted) and previous outbreaks have been associated with the consumption of vegetables (Launders et al., 2016b), salad (Sinclair et al., 2017), and herbs (Cowley et al., 2016). Certain clades are endemic in the United Kingdom cattle population (Food Standards Agency (FSA), 2018), while other clades are more commonly isolates from cases reporting recent travel to other countries in Europe and beyond (Figure 4). The phylogenetic placement of the outbreak strain indicated it belonged to a travel-associated (i.e., non-United Kingdom) clade. As none of the cases linked to the outbreak reported recent travel outside the United Kingdom, this provided circumstantial evidence that the contaminated vehicle may have been imported food. The

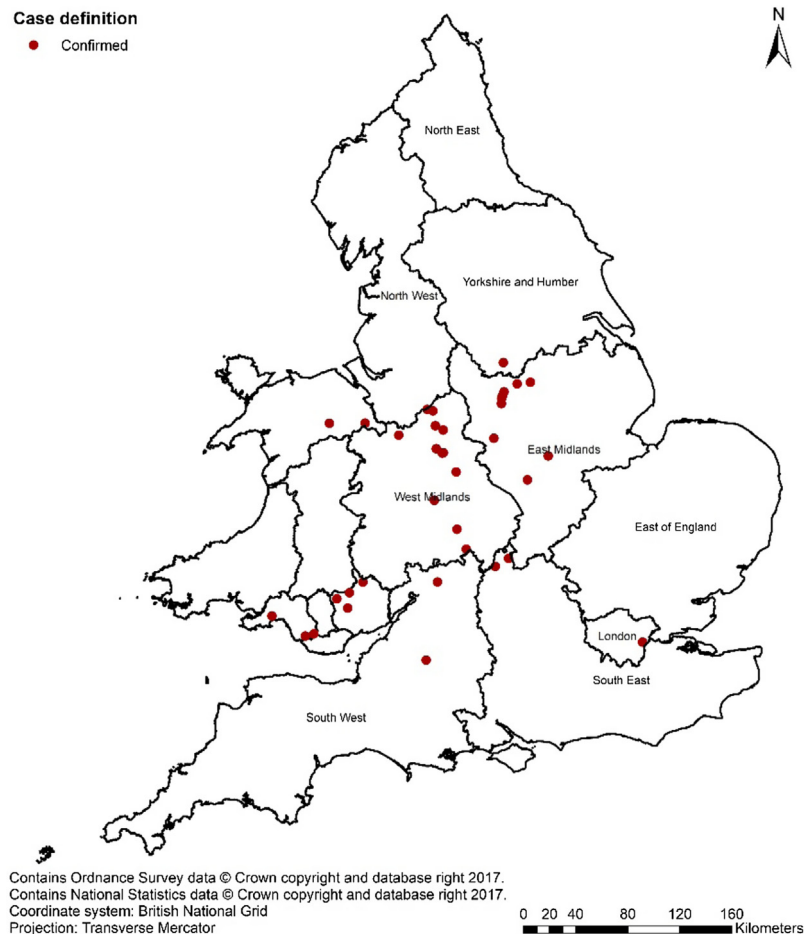


Figure 3. Geographic spread of outbreak cases by resident postcode in England and Wales; Shiga toxin-producing *Escherichia coli* O157:H7 outbreak, United Kingdom, August 2020.

Table 1

Single variable and multivariable model for the case–case study; outbreak of Shiga toxin-producing *Escherichia coli*, United Kingdom, August 2020.

	Cases n (%)	Comparisons n (%)	OR (95% CI)	aOR (95% CI)	P-value ^a
Fast-food product X	7 (26%)	0 (0%)	36.01 (5.12–∞)	31.8 (1.62–625)	0.023
Indian takeaways	7 (26%)	4 (5%)	6.65 (1.77–24.98)	6.99 (1.16–42.3)	0.034
Raw vegetables ^b	15 (56%)	20 (25%)	3.75 (1.51–9.34)	2.24 (0.93–11.3)	0.066
Iceberg lettuce	10 (37%)	12 (15%)	3.33 (1.23–9.00)	2.35 (0.65–8.58)	0.195

aOR, adjusted odds ratio; OR, odds ratio; CI, confidence interval.

^a Wald P-values from multivariable analysis.

^b This comprised a wide range of different vegetables, including (but not exclusively) peppers, onions, broccoli, lettuce (unspecified), salad (unspecified), cucumber, cabbage.

interrogation of EnteroBase identified the closest international match as a sequence from a Dutch cattle isolate sampled in 2007 at a SNP distance of 75 SNPs. Fifteen countries responded to the urgent query on EPIS, with none reporting recent increases in STEC O157:H7 *stx1+* *stx2* or any related strains.

Food sampling

Eleven samples were obtained from fast-food outlets and one retailer reported by a case (both from August 18) and tested; these included shredded lettuce, cucumber, and breaded/pre-cooked chicken (including the ingredients of the implicated fast-food product X from one restaurant of restaurant chain A). Additionally, loose supermarket peppers were tested from the home of one case (on August 24). All of these samples tested negative for *E. coli* O157.

Food chain investigations

The FSA contacted restaurant chain A to request information on their food supply chain, food production processes, and food safety management systems. The restaurant chain reported that both raw materials and finished product underwent regular microbiological testing and that all product was negative. Additionally, this item (as well as several other items) had only been re-added to the menu in the week prior to the outbreak detection. The majority of the implicated restaurants from restaurant chain A were supplied by a single distribution centre in the Midlands region. The cucumber supplied to their restaurants via this distribution centre was grown in the Netherlands by a corporation of 11 growers co-ordinated by company G, and distributed to the United Kingdom through

Table 2

Single variable analysis focusing on a priori hypotheses: consumption of food at restaurant chain A, consumption of product X, and/or consumption of cucumber; outbreak of Shiga toxin-producing *Escherichia coli*, United Kingdom, August 2020.

Study population	Variable	Cases n/N (%)	Controls n/N (%)	OR (95% CI)	P-value
All cases + controls (n = 110)	Ate the fast-food product X	10/25 (40%)	2/85 (2%)	27.7 (4.95–271)	<0.001
All cases + controls (n = 106)	Ate the fast-food product X and/or cucumber separately	23/25 (92%)	28/81 (36%)	21.8 (4.69–198)	<0.001
Only those that ate at restaurant chain A (n = 28)	Ate the fast-food product X	10/13 (77%)	2/15 (13%)	21.7 (2.35–265)	<0.001
All cases + controls (n = 106)	Ate cucumber	22/25 (88%)	26/81 (32%)	15.5 (4.02–85.8)	<0.001
All cases + controls (n = 110)	Ate out	23/25 (92%)	42/85 (49%)	11.8 (2.59–107)	<0.001
Only those that did not eat at restaurant chain A (n = 82)	Ate cucumber	10/12 (83%)	22/66 (33%)	10.0 (1.84–98.6)	0.001
All cases + controls (n = 110)	Ate at restaurant chain A	13/25 (52%)	15/85 (18%)	5.06 (1.71–14.7)	<0.001
Only those that had eaten out (n = 65)	Ate at restaurant chain A	13/23 (57%)	15/42 (36%)	2.34 (0.73–7.53)	0.105

OR, odds ratio; CI, confidence interval.

Table 3

Single variable and final multivariable model for the case-control study; outbreak of Shiga toxin-producing *Escherichia coli*, United Kingdom, August 2020.

	Cases n (%)	Controls n (%)	OR (95% CI)	aOR (95% CI)	P-value ^a
Fast-food product X	10 (40%)	2 (2%)	27.7 (4.95–271.21)	9.19 (1.02–82.8)	0.048
Cucumber ^b	22 (88%)	26 (32%)	15.5 (4.02–85.8)	5.84 (0.24–141.03)	0.277
Ready meal from any supermarket	7 (28%)	5 (6%)	6.22 (1.47–27.34)	5.40 (0.71–41.02)	0.103
Italian restaurant	5 (20%)	3 (4%)	6.83 (1.19–46.59)	4.98 (0.18–21.77)	0.800
Eating tomato outside the home	12 (48%)	7 (9%)	9.76 (2.84–34.38)	4.20 (0.65–27.05)	0.131
Any lettuce	24 (96%)	37 (46%)	28.5 (4.14–1199.47)	3.64 (0.10–127.24)	0.477
Age			Linear function	0.898 (0.844–0.956)	0.001
Female sex	15 (64%)	46 (54%)	1.51 (0.55–4.31)	0.941 (0.136–6.500)	0.951

aOR, adjusted odds ratio; OR, odds ratio; CI, confidence interval.

^a Wald P-values from multivariable analysis.

^b Consumption either inside or outside the home.

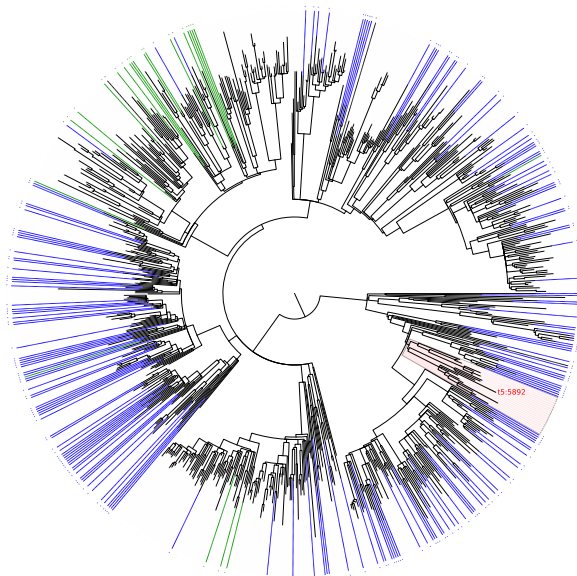


Figure 4. Maximum likelihood phylogeny of CC11-lineage IIc complex coloured by travel (blue) or animal origin (green), from the outbreak of Shiga toxin-producing *Escherichia coli*, United Kingdom, August 2020. The monophyletic clade where outbreak strain t5:5892 clusters is highlighted in pink.

company F, as well as exported to other European countries (Figure 5). The implicated fast-food restaurant chain A immediately stopped using all the cucumber growers that had been identified on first notification of the outbreak and removed all cucumbers from restaurants supplied by the Midland's distribution centre.

Discussion

Here, we describe an outbreak of STEC O157 that occurred in the United Kingdom in August 2020. This study emphasizes how phylogenetic data were able to directly guide outbreak investigations, through comparison of existing evidence on the outbreak sub-lineage and clade (sub-lineage IIc is more frequently associated with outbreaks from fresh produce, and with data available on EnteroBase we were able to link the strain to the Netherlands).

Epidemiological investigations provided rapid and strong evidence that a vehicle of infection was a specific fast-food product (product X) from a restaurant chain (restaurant chain A), and the most likely cause of the contamination was through imported cucumbers, providing further evidence that salad items are an important vehicle of STEC outbreaks. This implicated fast-food product had recently been re-added to the menu, as the chain had previously only offered a limited menu due to COVID-19 restrictions. The suspected batch of cucumbers was immediately discarded on notification of the outbreak to the retailer and no

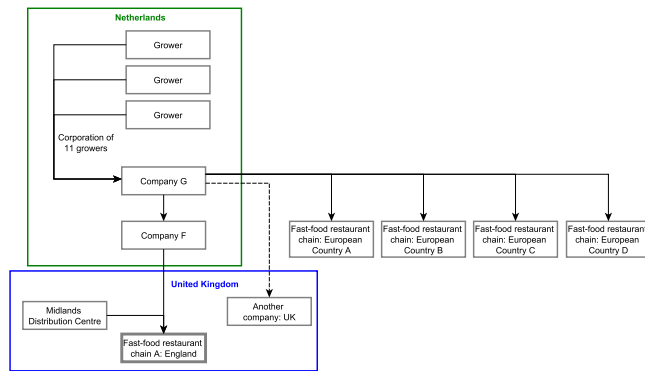


Figure 5. Supply chain for cucumber used in fast-food product X from restaurant chain A (the dashed line indicates an indirect supply chain).

further cases were identified. Strong communication links with the restaurant chain, European Union (via RASFF), and Dutch authorities enabled the swift and in-depth analysis of food chains. These timely interventions led to an immediate reduction in risk and outbreak control. Together, this report highlights the good practice with respect to early detection of the outbreak based on local exceedances, combined with ESQs, which provided an early indication that eating out at restaurant chain A was a common exposure among cases.

One noteworthy aspect of this outbreak is that the contaminated vehicle was fresh produce, raising multiple challenges. For instance, patients often neglect to mention salad items in their food histories. This was observed in the case–control study, where individuals who ate cucumber-containing items did not report having eaten cucumber, and this may have led to an underestimation or obscuring of further effects. Secondly, the short shelf-life of these items means that the contaminated food items are often not available for testing during outbreak investigations. In this outbreak, while ingredients from fast-food product X were tested 2 days prior to the cucumber batch being discarded, no STEC contamination was detected. However, such negative microbiological findings in implicated food products (especially in salad products) are not uncommon, as STEC can be reportedly difficult to isolate from implicated food due to the infectious dose often being below that detected by standard testing (Byrne et al., 2016). For instance, in a United Kingdom-based outbreak linked to salad leaves, despite large numbers of salad leaves tested, the only samples positive for the outbreak strain came from the irrigation water (which could be filtered and therefore concentrated before testing) (Jenkins et al., 2015). In another United Kingdom-based outbreak linked to unpasteurized milk, the outbreak strain was only successfully detected in cattle faecal samples (Treacy et al., 2019). This emphasizes the importance of proactive sampling and surveys of cattle/sheep to obtain microbiological confirmation that nearby animal reservoirs are colonized with the outbreak strain (Söderström et al., 2008), and reinforces the importance of robust epidemiological and food-tracing evidence. Finally, despite the food business operators testing for *E. coli*, it was not detected prior to or during this outbreak, which questions the meaningfulness and utility of post-harvest/pre-supply testing. Additionally resistance to sharing adequate details of complex supply chains from food business operators limited and confounded the trace-back efforts.

There were several limitations to the epidemiological studies that are important to note. The case–case analysis used data previously collected and held in NESSS and as such, some of the variables were not ideal for the purpose of this investigation: e.g. ‘raw vegetables’ could include any salad or vegetable items and this made it difficult to identify certain items of interest or test wider

consumption. As with all case–case analyses, the use of non-outbreak ‘comparison’ cases may have resulted in over-matching of exposures with outbreak cases, reducing our ability to detect risk factors for infection. However, the speed of the case–case study (analysis within approximately 48 h following the outbreak report) enabled us to test the primary hypotheses at pace and guided rapid public health action, which may not have been achieved through alternative study designs.

The decision to proceed with a case–control study added to the strength of evidence but at the expense of time. This is likely to have led to recall bias in both cases, whose food exposures were 6–8 weeks prior, and controls, who were asked about food exposures during a particular week 2 months prior in order to cover a similar time period to cases and take account of the impact of a government scheme encouraging people to eat out during August 2020. Additionally, the exposure period for controls was restricted to a single week – August 3 to August 9 – despite the symptom onset of cases being over August 3 to August 16; this may have led to some under-reporting if cases/controls had differing food exposures in the period August 9–16. Despite these limitations, this case–control study is a further example of the success in using market research panel-generated controls, allowing for a rapid response and requiring low resource, to aid rapid and cost-efficient results to guide outbreak management.

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Ethical approval

Not applicable.

Conflict of interest

None.

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